6. *Post-operative evaluation of patients*

Computerized tomography and magnetic resonance scans should be carried out at 7, 30, 90, 180 and 365 days after PDT. Any recurrence must be measured antero-posteriorly and latero-laterally; the data must be correlated with the neurological check-up and Karnofsky grading. If decompressive craniectomy has been performed during or after operation, it would be useful to describe the possible swelling of the flap.

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Topical application of liposomes

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1. *Introduction*

Liposomes are microscopic vesicles formed by amphipathic liquid membranes. They are used as models for biological membranes and have recently been exploited as delivery vehicles for systemic administration of drugs. Of particular interest to the photobiologist is their topical use. They are included in cosmetics and yet discounted as therapeutically ineffective when applied to the skin surface. In fact, liposomes are applied topically for different purposes and their effectiveness depends on their penetration and mechanism of drug delivery.

2. *Barrier function of the skin*

The barrier function of skin resides entirely in the outermost layer called the stratum corneum [1]. Penetration through the stratum corneum to the nucleated epidermis is passive, based on diffusion. The barrier to diffusion is due to dead keratinized squames derived from keratinocytes embedded in an intercellular matrix of lipid and aqueous channels which form broad lipid bilayers retaining water [2]. Thus stratum corneum contains hydrophilic and lipophilic compartments which act as an interdependent buffer to retard both water loss and also adsorption of aqueous material [3]. The strategy in designing topical liposomes is to exploit adsorption through the interface of these two compartments [4].

3. *Liposome structure*

The amphiphilic lipids which form liposomes contain head groups and paired, often saturated, lipid tails. At sufficient concentration in water the hydrophilic head groups orient outward and the hydrophobic lipid tails huddle together forming a bilayer sheet. The liposome sphere is the energetically favored three-dimensional form of these sheets. Liposomes are classified as unilaminar if they are composed of a single membrane or multilaminar if the membranes are layered like onion skin. Small liposomes are less than 0.05 μm in diameter.

Phospholipids are widely used in liposomes, and their head groups may be positively or negatively charged or neutral. Alternatively, charged species such as stearylamine or dicetyl phosphate can be added to impart a net charge to the membrane. The membrane charge is important for encapsulating charged compounds, and it may have an influence on liposome interaction with the target.

The lipid bilayer passes from a "gel" phase to a "liquid crystal" phase at a critical phase transition temperature (T_c) when the head groups become fully hydrated and the lipid chains are freely mobile. The length and saturation of the paired lipid chains determine the T_c value; lipids such as phosphatidyl choline form bilayers with T_c below room temperature, whereas dipalmitoyl phosphatidylcholine bilayers have a T_c value of 45 °C. This liquid crystal state is essential for liposomes to interact simultaneously with the lipid and aqueous compartments of the stratum corneum.

Cholesterol is frequently added to liposomes to enhance stability of the membrane. Liposomes have been made sensitive to pH changes by using phospholipids which form bilayers when charged but fail to stack when neutralized by a change in pH.

4. *Liposomes delivered extracellularly*

Empty liposomes hydrate the skin by simply contributing lipids to the stratum corneum [5], and cosmetics containing liposomes rely on this effect.

Liposomes contain domains for encapsulating active drugs. Lipophilic compounds can be integrated into the liposome membranes. Proteins, such as antibodies, have been embedded in the outer membrane after formation to target liposome delivery in systemic use. Water-soluble agents can be captured in the internal aqueous spaces between membranes and in the core.

Nearly all reports of topical liposome delivery have used liposomes which are designed to sequester drug in the lipid compartment of the stratum corneum and slowly release active species into the skin by dissolution of the liposomes. Mezei and Gulasekharam [6, 7] were the first to report that the corticosteroid triamcinolone encapsulated in liposomes and applied topically to rabbit skin was more concentrated in epidermis than after "free" drug application. A similar effect on human skin was reported by the same laboratory using the anesthetic tetracaine [8] and by another laboratory using hydrocortisone [9]. Liposomally entrapped methotrexate applied topically has been reported to be retained 2–3 times longer in mouse skin than the drug in free form [10]. Liposomes have also been used to encapsulate enzymes which inactivate toxins extracellularly [11], and have been proposed as a method for delivering sunscreens into the stratum corneum [12]. All these active species exert their effect extracellularly and the workers attribute the effectiveness to sequestration and timed release into the epidermis.

These findings have been vigorously contested. Radiolabeled lipids incorporated into liposomes similar to those of Mezei and Gulasekharam have been reported to remain entirely on the surface of guinea pig skin and do not penetrate the stratum corneum [13]. Liposomes have been reported to be unable to pass completely through full-thickness mouse skin, and no evidence has been found that they fuse with the stratum corneum [14]. No advantage has been found in topical application of the androgen dihydrotestosterone encapsulated in liposomes compared with the drug in acetone [15]. The reports of enhanced drug delivery by liposomes have been attributed to the lipid film produced on the skin, which increases hydration [5].

5. *Liposomes delivered intercellularly*

Liposomes deliver their contents to the cytoplasm of cells in culture either by fusion with the outer cell membrane or by endocytosis, whereupon they are concentrated in acidic lysozymal sacs [16]. This method of delivery is generally regarded as inefficient in animals and humans because only reticuloendothelial cells efficiently take up liposomes [16].

However, our laboratory has developed a method to deliver active enzymes efficiently to skin cells both in culture and in animal skin. We have focused on the delivery of the DNA repair enzyme T4 endonuclease V which initiates removal of pyrimidine dimers in DNA produced by UV exposure. The active enzyme was encapsulated in several types of liposomes, and DNA repair was increased when they were added to both human fibroblasts and keratinocytes [17]. A pH-sensitive liposome containing the DNA repair enzyme increased DNA repair in UV-irradiated keratinocytes from human skin explants [18], and enhanced removal of pyrimidine dimers from the epidermal keratinocytes of UV-irradiated hairless mouse skin within 6 h after topical application in a lotion form [19]. This represents the first demonstration of delivery of active enzyme into skin cells. This liposome preparation received Orphan

Drug status from the U.S. FDA for treatment of patients with the rare human genetic disease xeroderma pigmentosum, who are deficient in repair of UV-induced DNA damage, and suffer from an extraordinarily high incidence of skin cancer on sun-exposed skin.

6. *Summary and prospects*

Topically applied liposomes penetrate the stratum corneum by exploiting the lipid–water interface of the intercellular matrix. Research has centered on empty liposomes or those containing drugs which dissolve extracellularly and release their contents to achieve high, localized concentration of lipid and/or drug. The positive reports in the literature have been disputed.

For photobiologists, topical application of photosensitizers may be enhanced by encapsulation in liposomes. Higher and longer-lasting drug concentrations may be produced in localized areas of skin, particularly at disease sites where the stratum corneum and the skin barrier function are disrupted. The liposome membrane should be designed to capture lipophilic drugs in the membrane or hydrophilic drugs in the interior. There is a strong burden of proof to demonstrate liposome penetration and the superiority of liposomes over traditional preparations followed by lipid overlays.

A novel method to topically deliver active enzymes from liposomes into epidermal cells has been developed to enhance DNA repair of UV-irradiated skin. This technology opens up a new field of correcting or enhancing skin cell metabolism to improve human health.

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